collars limits their use to some extent" (*Lancet*, 1958). It has also been suggested that conservative treatment should not include the prolonged use of collars, because prolonged complete immobilization allows neck muscles to atrophy or become lax (Allen, 1952).

Both these views are misguided. A patient who has signs of progressive degeneration of the spinal cord with the prospect of quadriplegia should not be deterred, by self-consciousness about his appearance, from timely immobilization in a light Minerva type of collar if this has a good chance of halting or reversing the disease. While such a collar gives more complete immobilization than a short "under the chin" model, it does not allow the muscles to atrophy any more than does a walking-plaster on a limb. The patient remains ambulant, and can in many cases return to work in his collar.

Such a collar has, we believe, allowed striking improvement in a number of our severely disabled cases, and should be used more often. It should be used reasonably early. Good results cannot be expected where symptoms have been present for over a year. It is impracticable where the patient is bedfast. In one or two cases where an otherwise disabled patient has been able to sit up, the Zimmer type of traction has been a useful temporary expedient. Otherwise we have not found traction helpful.

While cases of prolapsed nucleus pulposus require prompt operation and should be recognized, satisfactory recovery from severe neurological disability in myelopathy from cervical spondylosis can occur with a conservative regime. The principle of rest is easier to understand and apply than operative measures. It also has obvious advantages where the diagnosis may remain in doubt.

Summary

The differential diagnosis, treatment, and prognosis of 60 cases with cervical disk lesions followed up for periods of two to ten years are reviewed.

The importance of differentiating the various types of disk lesion and concomitant spinal cord and other disorders is stressed, as is the necessity of a long follow-up.

Improved results in cases of cervical spondylosis with myelopathy have been associated with a conservative regime, and in particular the use of a light Minerva collar.

We thank Mr. G. L. Alexander and Mr. A. Hulme, who have kindly allowed cases under their care to be included in this survey; and Dr. H. L. Hoffman, who has been associated with the treatment of some of these cases.

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GENETIC CONTROL OF ISONIAZID METABOLISM IN MAN

BY

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Isoniazid (l-*iso*nicotinyl hydrazide) was first synthesized by Meyer and Mally in 1912, but its chemotherapeutic value was not discovered until forty years later. Grunberg and Schnitzer (1952) and Grunberg *et al.* (1952) showed that the compound was bacteriostatic against *Mycobacterium tuberculosis* strain H37Rv *in vitro*, and that it protected mice from developing tuberculosis when they were injected with tubercle bacilli intravenously. Robitzek *et al.* (1952) and Selikoff and Robitzek (1952) showed that the compound was effective in the treatment of human tuberculosis.

A considerable amount of work has been done on the metabolism of isoniazid both in animals and in man. These studies have shown that there is rapid and complete absorption of the drug, giving an initial high blood-level. When this falls the greater part of the compound appears in the urine within 24 hours of oral administration (Elmendorf *et al.*, 1952; Barclay *et al.*, 1953).

A large variation in the metabolism of isoniazid was found to exist among human beings by Bönicke and Reif (1953), Hughes (1953), Hughes *et al.* (1954, 1955). Distribution histograms of the percentage of the administered isoniazid which is excreted in the free form in the urine were given by Biehl (1956, 1957). These were bimodal, suggesting that individuals belong to one of two classes—either rapid or slow inactivators.

Mitchell *et al.* (1958) thought that this polymorphism for isoniazid metabolism in man might be genetically determined, and support for this view was afforded by the finding that groups of Japanese subjects contain a much larger proportion of rapid inactivators than groups of Caucasian subjects (Harris *et al.*, 1958).

To test the genetic hypothesis a study of 20 families was carried out by means of a microbiological method by Knight *et al.* (1959) and reported while the present study was in progress. They showed that it was probable that "slow inactivators" of isoniazid (who have high blood-levels of the free compound and excrete a high proportion of the free drug in the urine) were recessive to the "rapid inactivators," and, moreover, that the genes concerned were autosomal (see Table I).

The primary purpose of this paper is to report the results in a larger series of families (53) than that of

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TABLE I.—*Summary of Family Studies (Reproduced from the Paper of Knight et al., 1959)*

	No. of Marriages	No. of Children	Observed		Calculated		χ^2	*p* Value
			Rapid	Slow	Rapid	Slow		
Slow × slow ..	5	19	0	19	0	19	—	1·00*
Rapid × slow	5	19	11	8	15·6	3·4	6·02	0·015†
Rapid × rapid	10	36	17	19	20·8	15·2	1·24	0·25‡

* Good correlation between observed and calculated numbers.
† The correlation is not good.
‡ Good correlation between observed and calculated numbers.

Knight *et al.*, using in the investigation a more accurate biochemical method than the microbiological assay employed by them.

Secondary undertakings have been to find out whether there was a difference in the occurrence of the two phenotypes between Caucasians and American negroes on the one hand and tuberculous and non-tuberculous subjects on the other. It can also be speculated that this genetically determined character may have importance in antituberculous therapy. "Rapid inactivators" of isoniazid may respond less well when their tuberculous disease is treated with the drug, while "slow inactivators" may well be more prone to develop polyneuritis on isoniazid therapy.

Materials

Plasma was obtained from experimental subjects by withdrawing 25 to 30 ml. of blood, and clotting was prevented with approximately 0.3 ml. of heparin solution (1,000 units per ml. in physiological saline).

Experimental subjects were derived from the following sources: (1) White and American negro patients at the Loch Raven Veterans Administration Hospital, Baltimore. (2) Members of the professional, secretarial, and technical staff of the Johns Hopkins Hospital. (3) Healthy American negro prisoners at the Maryland House of Correction, Jessup, Maryland. (4) Caucasian family units which were derived in the following ways: (*a*) families on the files of the Medical Care Clinic of the Johns Hopkins Hospital; (*b*) families of professional and secretarial staff of the Johns Hopkins Hospital; (*c*) families of white patients at the Baltimore Veterans Administration Hospital for tuberculosis; (*d*) families of children in Eudowood Children's Sanatorium, Baltimore; (*e*) families of children on the Medical Care Files of the paediatric out-patient service at Baltimore City Hospital; and (*f*) twins and their families obtained by using the records of twin births in the obstetric department of the Johns Hopkins Hospital between 1942 and 1952.

Children under 5 years of age were usually excluded as experimental subjects, owing to the quantity of blood required at venepuncture.

Methods

The plasma-free isoniazid concentration was estimated by a slight modification of the method of Maher *et al.* (1957). The principle of this method is the extraction of the isoniazid from the plasma with an organic solvent, and estimating its condensation product with vanillin spectrophotometrically. Technical details will be reported elsewhere.

Where a subject was receiving drugs these were discontinued for 48 hours before the test procedure.

The in-patients at the Veterans Administration Hospital were given 9.8 mg. of isoniazid per kg. body weight orally in a single dose. A light meal of toast and coffee was allowed after the compound had been given. The blood sample was taken six hours after ingestion of the drug.

The same procedure was followed for family members. For the House of Correction healthy American negro subjects the dosage used was 10 mg. of isoniazid per kg. body weight.

The blood was stored at +4° F. (−15.6° C.), and if chemical analysis could not be performed in five days the plasma was stored at −20° C. until required.

Erythrocytes for blood grouping were sent to Dr. Fred H. Allen, jun., in Boston, for the purpose of classifying twinships, and eventually for linkage analysis.

Standard statistical methods were taken from Brownlee (1948). The means of the plasma isoniazid concentrations for various groups were compared by the procedure of Scheffé (1953), which circumvents the difficulties of multiple t-testing.

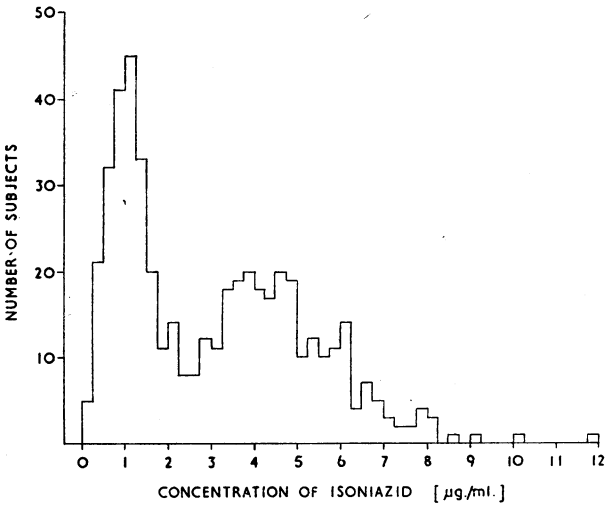

FIG. 1.—Plasma isoniazid concentrations six hours after drug ingestion. 484 persons were investigated. The results for 483 are shown. One result of 14.12 μg./ml. has been omitted. The dose of isoniazid which these subjects received varied between 9.8 and 10 mg. per kg. body weight.

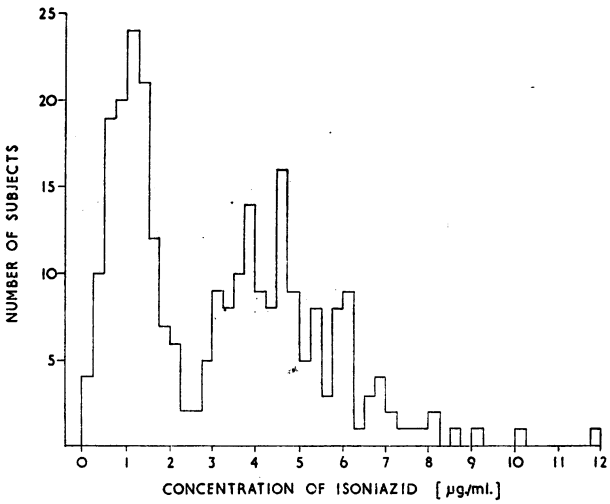

FIG. 2.—Plasma isoniazid concentrations six hours after drug ingestion. Results obtained in 267 members of the 53 complete family units investigated are shown. All these subjects received approximately 9.8 mg. of isoniazid per kg. body weight.

Results

The standard error of estimation of plasma isoniazid concentration was computed from an array of "standards" which was included in every experiment. It was found to be 0.224 μg./ml. over the range of concentration 0 to 5 μg./ml.

Determinations of plasma isoniazid six hours after drug ingestion were made on 484 persons. The frequency distribution-curve for these values is shown in Fig. 1. There is an antimode at 2.5 μg./ml. concentration. Persons who have plasma concentrations of isoniazid of less than 2.5 μg./ml. six hours after an oral dose of 9.8 or 10 mg. per kg. body weight are termed "rapid inactivators." Those with values above 2.5 μg./ml. are termed "slow inactivators."

Occurrence of Phenotypes in the Populations Studied.—When individuals were classified into "rapid" and "slow" inactivators of isoniazid, in the manner defined above, their occurrences in populations were found to be as indicated in Table II. Five Veterans Administration tuberculous white patients, whose families were studied, are included with the family parents, while four healthy American negro subjects are included with the Veterans Administration tuberculous American negro group.

TABLE II.—*Distributions of Two Isoniazid Inactivator Phenotypes in Different Populations*

Population Studied	Rapid Inactivators		Slow Inactivators		Totals
	No.	%	No.	%	
Unrelated white subjects (parents of families, etc.)	57	46·7	65	53·3	122
V.A. hospital tuberculous white subjects	39	50·0	39	50·0	78
V.A. hospital tuberculous American negro subjects	26	52·0	24	48·0	50
Maryland House of Correction healthy American negro subjects	17	41·5	24	58·5	41
	139	47·8	152	52·2	291

TABLE III.—*Summary of the 53 Caucasian Families*

Mating	No. of Families	No. of Children	Phenotypes of Children	
			Rapid Inactivator	Slow Inactivator
S×S	1	1	1	—
	1	1	—	1
	6	2	—	2
	2	3	—	3
	1	3	2	1
	1	4	—	4
	2	5	—	5
	1	5	1	4
	2	6	—	6
R×S	2	1	1	—
	2	2	2	—
	3	3	3	—
	1	4	4	—
R×S	2	1	—	1
	2	2	1	1
	1	2	—	2
	1	3	2	1
	1	3	1	2
	1	3	—	3
	2	4	3	1
	1	4	2	2
	2	4	1	3
	1	5	2	3
	1	6	4	2
R×R	1	1	1	—
	2	2	2	—
	3	3	3	—
	1	7	7	—
R×R	2	2	1	1
	2	3	2	1
	1	3	1	2
	1	4	3	1

Family Data

A summary of the 53 Caucasian families studied is shown in Table III. The results shown include the following three repeat tests.

Mr. W. K., aged 44, consumed sufficient alcohol to make him obviously drunk when the blood was taken. The estimation showed 2.77 μg. of isoniazid per ml. of plasma. Lest the alcohol should have interfered with the metabolism of the isoniazid, the test was repeated two weeks later, the blood being taken at 5.30 a.m. The second estimation showed 4.46 μg./ml.

Miss R. K., aged 7, mentally retarded, was observed to spit out some of the tablets. The plasma at six hours contained 2.03 μg. of isoniazid per ml. A second test was carried out two weeks later by passing a Levine tube into the stomach and pouring the correct dose of crushed tablets into water down the lumen. Six hours later the plasma contained 6.02 μg./ml.

Master J. R., aged 10 was found to be incorrectly weighed. He was given 200 mg. of isoniazid, and the plasma level was 2.48 μg./ml. After he was given the correct dose of 300 mg. the level of plasma isoniazid was 6.21 μg./ml.

There are two sets of monozygous twins in the 53 families. In Table III, and in all subsequent statistical analysis of the family data, a monozygous twinship is treated as a single individual.

TABLE IV.—*Results of Isoniazid Inactivator Phenotype Determination in Twinships*

	Type of Twinship	
	Monozygous	Dizygous
Concordant	5	4
Discordant	—	1

Twin Data

The 10 pairs of twins investigated yielded the results shown in Table IV.

Nine twinships are classed for zygosity according to blood-group data obtained on the two members. In the tenth the sexes of the two members of the twinship were different.

Statistical Analyses

Heterogeneity Tests

Absence of Heterogeneity Due to Race.—The occurrences of the two phenotypes in the two American negro populations studied are not significantly different from their occurrences in the American white populations. For the data in Table II $\chi^2 = 1.220$ with 3 degrees of freedom and hence $p>0.5$.

Absence of Heterogeneity Due to Tuberculosis.—The fact that the χ^2 value for Table II is not significant suggests that tuberculosis does not affect one phenotype more than the other. A more stringent test has been carried out by the method of Woolf (1955). The results indicate that rapid inactivators could be twice as liable to develop tuberculosis as slow inactivators without this showing on the present numbers.

Absence of Heterogeneity Due to Sex.—The influence of sex on the division of subjects into the two phenotypes has been studied in the white non-tuberculous population. The white tuberculous, American negro tuberculous, and prisoner populations are not suitable for this purpose as they contain only three, three, and no women respectively. The distribution of phenotypes by sex is shown in Table V. For these data, $\chi^2 = 0.543$ and $p>0.1$. There is therefore no sex effect on the division of a population into "rapid" and "slow" inactivator phenotypes.

TABLE V.—*Distributions of Isoniazid Inactivator Phenotypes in Both Sexes Amongst 122 Unrelated Subjects*

	Rapid Inactivators	Slow Inactivators	Totals
Male	31 (50%)	31 (50%)	62
Female	26 (43·4%)	34 (56·6%)	60
Totals ..	57 (46·7%)	65 (53·3%)	122

TABLE VI.—*Numbers of Each Isoniazid Inactivator Phenotype Found in Various Age-groups Amongst 291 Unrelated Adult Subjects*

Age Groups	Rapid Inactivators	Slow Inactivators	Totals
20–29 years	14 (42·5%)	19 (57·5%)	33
30–34 ,, ...	27 (48·2%)	29 (51·8%)	56
35–39 ,, ...	24 (40·7%)	35 (59·3%)	59
40–44 ,, ...	19 (52·9%)	17 (47·1%)	36
45–49 ,, ...	13 (40·6%)	19 (59·4%)	32
50–59 ,, ...	23 (67·6%)	11 (32·4%)	34
60–79 ,, ...	19 (41·4%)	22 (58·6%)	41
Totals ..	139 (47·8%)	152 (52·2%)	291

χ^2=8·001. D.F.=6. $p>0·1$.

TABLE VII.—*Numbers of Each Isoniazid Inactivator Phenotype Found in Various Age-groups of Subjects of 20 Years of Age or Less*

Age Group	Rapid Inactivators	Slow Inactivators	Totals
0–8 years	23 (50·0%)	23 (50·0%)	46
9–12 ,, ...	34 (58·6%)	24 (41·4%)	58
13–16 ,, ...	20 (48·8%)	21 (51·2%)	41
17–20 ,, ...	5 (29·4%)	12 (70·6%)	17
Totals ..	82 (50·6%)	80 (49·4%)	162

χ^2=4·607. D.F.=3. $p>0·1$.

Absence of Heterogeneity Due to Age.—The influence of age on the division of the unrelated subjects into the two phenotypes has been studied and the results are shown in Table VI. Subjects of 20 years of age or less are presented in the same way in Table VII. Many of these are related. Two pairs of monozygotic twins are each treated as a single individual. It would seem, therefore, that age is without effect on the occurrence of the two phenotypes.

Variations of Plasma Isoniazid Concentrations with Variations in Weights.—It was found that, despite a very large scatter of observations, there is a significant correlation between weight and plasma isoniazid concentration in both phenotypes. The significance of the regression of the latter on the former is much greater for the slow than for the rapid inactivators. Speculation can be made concerning the reason for these correlations. It may be that weight is an inefficient index of the metabolism of isoniazid, and that small subjects metabolize the compound relatively faster than large subjects. Therefore it is possible that surface area or "metabolically active mass" (Drabkin, 1959) would be a better criterion on which to assess the dosage of the drug. The existence of this variability in the plasma isoniazid concentration due to variations in weight indicates another component in the variances of the distribution curves for the two phenotypes given in Figs. 1 and 2.

Variations of Plasma Isoniazid Concentrations with Variations in Dosages.—The "bridge" of values about the antimode is seen to be much higher in Fig. 1 than in Fig. 2. In the former histogram there are represented the results from 484 subjects who had received dosages of isoniazid varying between 9.8 and 10 mg. per kg. of body weight. The latter histogram shows the results from 267 family members, and all these subjects had been given dosages of approximately 9.8 mg. per kg. of body weight. This standardization of dosage reduces the variance of the distribution curves of the two phenotypes, and so reduces the numbers of persons in the antimodal region where the tails of the two curves overlap.

Examination of the Family Data

Consider as an hypothesis that human metabolism of isoniazid is controlled by two allelic genes and that the "slow inactivator" individual is an autosomal homozygous recessive.

Since 152 out of 291 subjects are slow inactivators the occurrence of this phenotype is 52.233677% of the population. As there is no heterogeneity between American negroes and whites, all unrelated subjects are included to estimate allele frequency. The frequency of the recessive allele (I_r) = $\sqrt{0.52233677}$ = 0.7227 (= p) and hence q = 0.2773 (I_r).

The hypothesis that "slow inactivation" is the homozygous recessive character was tested in two ways: (1) The observed matings have been compared with those expected by application of the Hardy–Weinberg law (Stern, 1943), as demonstrated in Table VIII. (2) The numbers of children of each phenotype expected have been calculated and compared with those observed as shown in Tables IX and X.

χ^2 = 0.093 (Table IX), and therefore with two degrees of freedom p>0.95.

A more rigorous method of testing this hypothesis is that given by Taylor and Prior (1939). This calculation gives χ^2 = 0.435, and therefore with 2 degrees of freedom

TABLE VIII.—*Numbers of Observed Matings Compared With Those Expected by Application of the Hardy–Weinberg Law*

Phenotypic Matings	Genotypic Matings	Expected Frequency of Matings		Expected Occurrence in 53 Matings	Observed Occurrence
S×S	$I_rI_r \times I_rI_r$	p^4 0·2728		14·46	17
R×S	$I_RI_R \times I_RI_r$	$2p^3q^1$ 0·0803	0·4990	26·45	23
	$I_RI_r \times I_RI_r$	$4p^3q$ 0·4187			
R×R	$I_RI_R \times I_RI_R$	q^4 0·0059			
	$I_RI_R \times I_RI_r$	$4q^3p$ 0·0616	0·2281	12·09	13
	$I_RI_r \times I_RI_r$	$4q^1p^3$ 0·1606			
		0·9999		53·00	53

I_R=The allele controlling the dominant character. χ^2=0·964. D.F.=2. $p>0·5$.

TABLE IX.—*Expected Numbers of Children of Each Phenotype Compared with Those Observed*

Phenotypic Matings	No. of Matings	No. of Children	No. of Children of Each Phenotype				χ^2	D.F.
			Rapid		Slow			
			Exp.	Obs.	Exp.	Obs.		
S×S	17	54	Nil	4	54	50	—	—
R×S	23	67	38·88	40	28·10	27	0·075	1
R×R	13	38	31·30	31	6·68	7	0·018	1
	53	159		75		84	0·093	2

The hypothesis is made that slow-inactivator persons are genetically homozygous recessives. The repeat determinations are not employed in this table.

TABLE X.—*Expected Numbers of Children of Each Phenotype Compared with Those Observed*

Phenotypic Matings	No. of Matings	No. of Children	No. of Children of Each Phenotype				χ^2	D.F.
			Rapid		Slow			
			Exp.	Obs.	Exp.	Obs.		
S×S	16	51	Nil	Nil	51	51	—	—
R×S	24	70	40·62	42	29·36	28	0·110	1
E×R	13	38	31·30	31	6·68	7	0·018	1
	53	159		73		86	0·128	2

In this table the repeat values have been employed. The expected numbers of children are calculated on the hypothesis that slow-inactivator subjects are genetically homozygous recessives.

$p>0.5$. This indicates that the observed numbers of segregating and non-segregating families are not significantly different from those expected. Hence, by this method also, the data fit the hypothesis that the slow-inactivator phenotype represents the genetically homozygous recessive individual.

Four individuals, including one of the three in whom a repeat test was performed as indicated under Family Data, above, were subjected to retest because their phenotypic status appeared incongruous in their family setting when viewed in the light of the hypothesis that "slow inactivation" is the recessive trait. In each case congruous findings resulted from the second test. The results of this *ex post facto* correction are shown in Table X. In the critical mating type, slow × slow, no rapid-inactivator offspring were observed.

When the family data are examined by the same two methods on the hypothesis that "rapid inactivation" is the homozygous recessive, the agreement of observed with expected was found to be unsatisfactory.

Examination of the Family Data for a "Dosage" Effect of the Allele Controlling the Dominant Character

Since the genetic analysis indicates that rapid-inactivator subjects may be genetically of two types—heterozygous and homozygous dominants—it is of interest to see if any difference can be detected between these two groups of people. Three things make this a difficult task. Firstly, although heterozygotes can be recognized from their positions in family pedigrees, there is no certain way of recognizing homozygous dominant individuals. Secondly, the variability in the plasma isoniazid concentrations in the rapid-inactivators group as a whole is relatively small. Thirdly, the variation in plasma isoniazid concentration associated with variation in weights must be controlled in the investigation.

By examination of the 53 pedigrees, 28 heterozygous parents and 42 heterozygous offspring are recognizable. The remainder of the rapid inactivators in the 53 pedigrees are a mixture of heterozygous and homozygous dominant individuals; in this category there are 22 parents and 33 offspring. The mean plasma isoniazid concentrations and the standard errors of the means are shown in Table XI.

A significant difference cannot be demonstrated between the means of the weight of (1) the heterozygous and other rapid-inactivator parents, (2) the heterozygous and other rapid-inactivator offspring, or (3) all heterozygotes and all other rapid inactivators.

The means of the plasma isoniazid concentrations can therefore be compared in these groups without danger of interference by differences in their weights.

The izoniazid concentrations have been subjected to the analysis of variance technique. This is shown in Table XII.

There is thus a much larger variability of plasma isoniazid concentration between groups than there is within groups. The means of the two groups of parents and offspring have been compared by the method of Scheffé (1953). The results of this comparison are shown in Table XIII.

There is shown to be a significant difference at the $p = 0.01$ level between the mean plasma isoniazid concentrations of "all heterozygotes" and "all other rapid inactivators." The latter group contains both heterozygous and homozygous individuals, and therefore its mean value is probably higher than would be the mean value for a group composed entirely of homozygous subjects. The fact that a difference can be demonstrated is therefore good evidence for the presence of a "dosage" effect due to the possession of two alleles controlling the dominant character, as compared with one.

TABLE XI.—*Data for Family Members Who are Recognizable as Heterozygotes, and Other Rapid-inactivator Family Members*

	Plasma Isoniazid Concentration (μg./ml.)		
	No. of Observations	Mean	Standard Error of Mean
Heterozygote parents	28	1·441	0·0906
,, offspring ..	42	1·149	0·0825
Other rapid-inactivator parents	22	1·072	0·0875
Other rapid-inactivator offspring	33	0·798	0·0756
All heterozygotes	70	1·2661	0·0633
All other rapid inactivators	55	0·9080	0·0597

TABLE XII.—*Analysis of Variance of Plasma Isoniazid Concentrations of Rapid-inactivator Family Members Divided into Parents and Offspring, and Heterozygotes and "Others"*

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Squares
Between groups ..	6·3738	3	2·1246
Within ,, ...	27·5072	121	0·2273

$$F \text{ ratio} = \frac{2{\cdot}1246}{0{\cdot}2273} = 9{\cdot}347. \quad D.F. = 3, 121. \quad p = 0{\cdot}001.$$

TABLE XIII.—*Comparisons of Means of Plasma Isoniazid Concentrations (μg./ml.) of Proved Heterozygotes and Other Rapid-inactivator Family Members*

	Level of Probability	Difference Between Means (μg./ml.)	Confidence Limits of the Difference	
			Lower	Upper
Heterozygote parents and other rapid-inactivator parents	0·05	0·369	−0·017	+9·755
Heterozygote offspring and other rapid-inactivator offspring	0·05	0·351	+0·035	+0·667
	0·01		−0·119	+0·821
All heterozygotes and all other rapid-inactivators ..	0·01	0·358	+0·060	+0·656
	0·001		0·000	+0·716

Twin Data

The number of twinships studied is small (Table IV), but the presence of a discordant twinship in the dizygotic group and not in the monozygotic group is evidence in favour of the genetic control of isoniazid metabolism by at least two segregating alleles.

Discussion

There must be factors operating which account for the presence of the large variances for the plasma isoniazid concentrations in both the rapid and slow inactivator groups (see Figs. 1 and 2). One such factor—namely, the variations in plasma isoniazid concentrations related to variations in weight—has already been mentioned. Two other possible mechanisms which may contribute to the variances are now discussed.

Optical Densities of Plasma from Persons who had not Ingested Isoniazid

The optical density of the plasma in persons who have not received any drug is less than the equivalent of 1.5 μg. of isoniazid per ml. (Berte *et al.*, 1959). This factor must contribute to the variances of the two parts of the curves in Figs. 1 and 2. The probability of this factor causing a "rapid inactivator" to appear a "slow inactivator" is small.

Rate of Urinary Excretion of Free Isoniazid

Some 4 to 10% of the administered isoniazid is excreted in the urine in the free form within four hours

of ingestion, and some 5 to 15% within eight hours (Peters, 1958). The renal clearance for the compound (calculated as $\frac{\text{urinary excretion in } \mu\text{g./min.}}{\text{plasma concentration in } \mu\text{g./ml.}}$) ranged from 60 to 156 ml. of plasma/min. in the four subjects studied by Elmendorf *et al.* (1952). It is therefore likely that different rates of urine-flow reduce the plasma concentration of free isoniazid at different rates. This factor may well contribute to the variability in plasma isoniazid concentrations within the rapid and slow inactivator groups. No information has been obtained on this aspect of isoniazid elimination during the present study, and therefore its importance in the test procedure cannot be evaluated.

It seems likely, however, that variations in urinary excretion of isoniazid, like variations in non-isoniazid optical density, and in weight contribute to the total variance of the two curves in Figs. 1 and 2.

A clearer separation of the two phenotypes is highly desirable. It does not appear that much can be done to eliminate non-specific optical density. It would, however, be possible to administer doses calculated in terms of "metabolically active mass" weight 0.7 (Drabkin, 1959), and it might also be possible to administer standard amounts of liquid, calculated on the same parameter, during the test period. These procedures might enable the phenotypes to be more clearly separated.

Genetic Control of Isoniazid Metabolism

The family data presented above confirm the tentative conclusion of Knight *et al.* (1959) that the slow-inactivator person is an autosomal homozygous recessive. Rapid inactivators are therefore of two genotypes—heterozygotes and homozygous dominants.

The data clearly show the presence of a definite "dosage" effect of the allele controlling the dominant character in that the heterozygotes have significantly higher plasma isoniazid concentrations than the homozygous dominant subjects.

In view of the above facts, it may be presumed that the allele controlling the dominant character gives rise to a process which speeds the inactivation of isoniazid. This process is probably enzyme-mediated, and in view of the experiments of Hughes (already referred to) it may well consist in a facilitation or speeding of isoniazid acetylation.

Maintenance of the Polymorphism of the Isoniazid Inactivator Phenotypes

The reasons for the maintenance of nearly all human polymorphisms are unknown; some speculations can, however, be made concerning the factors responsible for the presence and maintenance in populations of the two isoniazid inactivator phenotypes. The metabolism of isoniazid *per se* is unlikely to have been such a factor in the past, though it may possibly become a significant one in the future. It is possible that there are naturally occurring compounds which are metabolized in the same way as isoniazid, and that these may possess antituberculous activity. The influence of such a class of compounds might be a mechanism whereby the recessive character might be preserved in populations. The data presented above contain insufficient numbers of tuberculous and non-tuberculous subjects to show whether or not there is a significant association of either phenotype with the development of tuberculosis.

Possible advantages of the dominant character are unknown. It is of interest that Harris *et al.* (1958), using a microbiological assay technique, have found that there is a much higher incidence of rapid inactivators among Japanese subjects than is present in European populations. Presumably, therefore, the dominant character is more advantageous in the Asiatic environment.

Therapeutic Implications of the Polymorphism of Human Isoniazid Metabolism

There are three aspects to the treatment of tuberculosis with isoniazid in which the inactivator phenotype of the patient might be of importance. These are (1) the development of polyneuritis with long-term exhibition of the drug, (2) the response of tuberculous disease to isoniazid treatment, and (3) the development of isoniazid-resistant tubercle bacilli.

Evidence bearing on the relationship of isoniazid inactivator phenotype to these topics is discussed below.

(1) The relationship of the pattern of isoniazid metabolism to the development of polyneuritis was investigated by Hughes *et al.* (1954). Seventeen tuberculous patients undergoing treatment were investigated. All except one received 20 mg. of isoniazid per kg. daily, while the exception received 10 mg./kg. The duration of treatment varied from 2 to 40 weeks. The average percentage of the daily isoniazid dosage recovered in the urine in the free form was calculated for each patient. These subjects can be classified for phenotype in the light of the results given by Biehl (1957) and shown in his Fig. 4. Persons with less than 12% recovery are termed rapid inactivators, persons with more than 16% recovery are termed slow inactivators, while those with values between 12 and 16% cannot be categorized. Of the 17 patients, according to these criteria, 10 are rapid inactivators, 5 are slow inactivators, and 2 cannot be categorized.

Polyneuritis occurred in 6 of the 17 subjects. Four of these six were slow inactivators and two were rapid inactivators. Hence four out of five slow inactivators developed polyneuritis, while 2 out of 10 rapid inactivators showed this complication of treatment. The rapid inactivators received rather longer courses of the drug.

The number of subjects involved is too small to draw any valid deduction, and the unequal lengths of the treatments also make it difficult to assess the results. There is a suggestion, however, that the slow isoniazid inactivators may be more prone than the rapid inactivators to develop polyneuritis.

(2) Mitchell *et al.* (1958) investigated the change of the sputum test for tubercle bacilli from "positive" to "negative" in 124 patients with cavitary pulmonary tuberculosis treated with isoniazid. The patients were phenotyped by determining their serum isoniazid concentrations, by a microbiological method, six hours after an oral dose of 4 mg./kg. Treatment was with a dosage of isoniazid of 12 mg./kg./day. Other antituberculous drugs were given to a majority of the patients. The results were as follows: (*a*) In 20 patients with moderately advanced disease, isoniazid inactivation had no effect on conversion. (*b*) In 41 patients with far-advanced disease bacteriological results were more favourable with "slow" than with the "intermediate" or "rapid" classification. (*c*) In 8 moderately advanced and 17 far-advanced cases there was some effect at the second month but none by the fifth. (*d*) In 11 moderately advanced and 26 far-advanced cases the differences were small and statistically insignificant.

It would therefore seem that the trend is for patients with the higher serum isoniazid concentrations to have a quicker reversal of infectiousness than those with lower serum concentrations. The frequency of reversal also tended to be rather higher in the "slow inactivator" (high-serum-isoniazid concentration) group. However, it must be pointed out that all these results are suspect because *para*-aminosalicylic acid

was administered simultaneously with isoniazid both in treatment and in the test to determine isoniazid inactivator phenotype. This substance interferes with the microbiological assessment of isoniazid concentration (Bell *et al.*, 1957), though the chemically determined concentration is not affected (Peters, 1958, 1959).

(3) Observations on the development of isoniazid-resistant strains of tubercle bacilli have been made by Biehl (1957). His patients have been phenotyped by the percentage of the daily dose of free drug administered, which is recovered in the 24-hour urine specimen. Less than 12% recovery corresponds to a rapid inactivator phenotype, more than 16% to a slow inactivator, and recoveries between 12 and 16% cannot be categorized. Biehl investigated two groups of patients. All had been under treatment for "well over six months." The results are summarized in Table XIV.

TABLE XIV.—*Development of Isoniazid-resistant Tubercle Bacilli in Patients Under Treatment with the Drug (Data from Biehl, 1957)*

Phenotype of Patients	Total No.	No. Developing Isoniazid-Resistant Organisms
Dosage 200 to 300 mg. Isoniazid Daily		
Rapid inactivator ..	46	14
Intermediate	9	3
Slow inactivator	24	7
	79	24
Dosage 20 mg. Isoniazid/kg. Body Weight Daily		
Rapid inactivator ..	17	3
Intermediate	1	1
Slow inactivator	25	5
	43	9

TABLE XV.—*Condensed Data of Biehl* (1957)

	Organisms		Totals
	Susceptible	Resistant	
Rapid inactivators ..	32	14	46
Slow ,, ...	17	7	24
Totals	49	21	70

The data from the first group have been submitted to the χ^2 test for heterogeneity in the form shown in Table XV. For these data $\chi^2 = 0.012$, and therefore with 1 degree of freedom $p>0.9$. Hence there is no significant heterogeneity. The inference is therefore that there is no association between the isoniazid inactivator phenotype and the development of tubercle bacilli resistant to the drug.

Extensive and controlled investigations are clearly required more accurately to define the role of the isoniazid inactivator phenotype in the therapy of tuberculosis.

It is hoped to carry out studies which will show unequivocally whether the isoniazid inactivator phenotype has an influence on the outcome of the subject's tuberculosis when the disease is treated solely with this drug. This is felt to be of considerable importance in view of the fact that great stress is laid on chemotherapy in underdeveloped countries (Dormer *et al.*, 1959), and in some of these the occurrence of the rapid inactivator phenotype may be much greater than in European populations. For example, Armstrong and Peart (1960) found that the great majority of Eskimos are rapid inactivators.

Summary

Plasma isoniazid concentrations have been determined chemically six hours after drug ingestion in 484 subjects. The frequency distribution curve of these concentrations is bimodal. Individuals are divided by the antimode into rapid and slow inactivators of isoniazid. There is no heterogeneity for these characters due to sex or age, and on the numbers studied the distribution of phenotypes appears to be the same in American negroes as in whites. The numbers of subjects studied are too small to determine whether tuberculosis affects one phenotype more than another.

267 of the subjects studied constitute 53 white families. The family data support the hypothesis that the slow-inactivator character is recessive. A "dosage" effect of the allele controlling the dominant character is demonstrable in that there is a significant difference between the mean plasma isoniazid concentration of recognizable heterozygotes and the mean value of all other rapid inactivators.

Possible reasons for the maintenance of the polymorphism are discussed, and so also are the therapeutic implications of this genetically determined isoniazid inactivator character.

We thank the following: Professor E. K. Marshall, jun., for suggesting this investigation; Dr. Patrick B. Storey, Director of Professional Services, Loch Raven Veterans Administration Hospital, Baltimore, for plasma from tuberculous patients; Dr. Fred H. Allen, jun., of Boston, for blood-grouping studies; Dr. J. M. Yosuico, physician to the House of Correction, Jessup, Maryland, for access to healthy American negro prisoners; Miss Margaret R. Hawkins and Mrs. Barbara J. Mitchell for social work among the families studied; Dr. Carmen F. Merryman and Mrs. Linda Ulenum for technical assistance; and Mrs. R. Kimmerer and Miss D. E. Rutledge for secretarial services. The manuscript was read by Dr. C. A. Clarke and Dr. E. B. Ford, F.R.S., and we are indebted to them for helpful suggestions concerning presentation.

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